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SRP-35 A Putative NAD(P)H Binding Protein Of Skeletal Muscle Sarcoplasmic Reticulum Membrane

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SRP-35 (sarcoplasmic reticulum protein of 35 kDa) is a newly identified integral membrane protein constituent of skeletal muscle sarcoplasmic reticulum. We have deduced the primary structure of the protein from cDNA clones isolated from mouse and rat skeletal muscle cDNA libraries. Primary sequence prediction analysis indicates that the NH2- terminal sequence of SRP-35 encompasses a transmembrane spanning segment or a signal sequence. In addition, SRP-35 is homologous to proteins belonging to the short-chain dehydrogenase/reductases family. Members of this protein family has two domains: the first involved in binding the nucleotide co-factor NAD(P)H, the second responsible for the catalysis of the substrate. SRP35 contains only a putative NAD(P)H binding site. Analysis of tissue distribution of SRP-35 by western blot analysis with affinity purified Ab shows that SRP-35 expression is specific for skeletal muscle since our Ab did not stain any protein in other tissues including heart, brain, liver, kidney, lung, spleen and stomach. Immunohistochemistry of primary cultured mouse myotubes transfected with SRP-35 EGFP construct indicates that SRP-35 is distributed on sarco(endo)plasmic reticulum membranes. Staining of western blot of sarcoplasmic reticulum membrane subfractions isolated from adult mouse skeletal muscle revealed that SRP-35 is associated with heavy sarcoplasmic reticulum. In addition, we found that SRP-35 is an integral membrane protein since it was extracted neither by $NaCO_3$ nor by high salt treatment of isolated sarcoplasmic reticulum membrane, but was solubilised by non-ionic detergent such as CHAPS, DDM and DHPC. We propose that , SRP-35 protein might provide the co-factor to enzymes involved in the generation of local reactive oxygen species within cellular subdomains

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ATP Release and P2X/P2Y Receptor Activation Account for Slow Calcium Transients Evoked by Electrical Stimulation in Skeletal Muscle Cells

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ATP released to the extracellular medium participates in cell signaling through activation of plasma membrane P2X (ion channels) or P2Y (metabotropic) receptors. Skeletal muscle cells express several P2X and P2Y receptor subtypes and ATP is profusely released during muscle activity. We have previously shown that depolarizing stimuli induce two calcium signals in skeletal myotubes: a fast signal associated with contraction and a slow signal that regulates gene expression. Here we show that extracellular nucleotides released by electrical stimulation are in part responsible for intracellular calcium signals. In rat myotubes, a tetanic stimulus (45 Hz, 400, 1 ms pulses) rapidly increased extracellular levels of ATP, ADP and AMP from 15sec to 3 min, with different half-life times. Exogenous ATP applications induced a dose-dependent increase in intracellular calcium, with an EC_{50} of 7.8 $\pm$ 3.1 μM. Exogenous ADP, UTP and UDP also promote calcium transients. By RT-PCR, we detected mRNA expression for $P2X_{1\text{-}7}$ and $P2Y_{1,2,4,6,11}$ in these cells. Both fast- and slow-calcium signals evoked by tetanic stimulation were partially inhibited by either 10-100 μM suramin (non selective P2X/P2Y blocker) or 2U/ml apyrase (nucleotidase that metabolizes ATP and ADP to AMP). In hemidiaphragm preparations, we demonstrated that apyrase reduces both twitch- and tetanus-evoked increase in tension. Our results suggest that nucleotides released during skeletal muscle activity act through P2X and P2Y receptors to modulate both calcium homeostasis and muscle physiology.

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Role Of K_{ATP} Channels During Fatigue And Metabolic Inhibition In Chicken Slow Skeletal Muscle

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The slow-twitch muscle fibers in contrast to the fast-twitch muscle fibers are fatigue-resistant. During fatiguing exercise, the ATP cost of contraction is reduced. This reduction in ATP can be sensed by K_{ATP} channels (ATP-sensitive potassium channels), which couple the metabolic state of the cell to its electric activity, causing its activation. Therefore, it has been proposed that K_{ATP} participate in fatigue in the fast-twitch skeletal muscle besides to ischemia in nervous system. In slow-twitch muscle fibers there are few studies related to muscle fatigue phenomena. Thus, in this study we designed an *in vitro* model for fatigue in chicken slow-twitch skeletal muscle to investigate the role of K_{ATP} channels in the fatigue process. We studied the effects of glibenclamide, which blocks K_{ATP} channels, on twitch and tetanus tension in the anterior *latissimus dorsi* slow muscle induced to fatigue. The results show that glibenclamide increases tension in the fatigued muscle. Also, the slow muscle was exposed to metabolic poisoning by cyanide, a condition in which the ATP formation is inhibited and when its intracellular concentration is diminished K_{ATP} channels are activated which in turn produces a reduction in muscle tension. The addition of glibenclamide in these conditions abolished the effect produced by cyanide. Moreover, we studied the possible role of intracellular calcium by studying the effects of glibenclamide on the contractures evoked by caffeine, which is known that releases calcium from sarcoplasmic reticulum. In these conditions glibenclamide increases tension. Thus, we are showing evidences of the role of K_{ATP} channels in the fatigue process, since glibenclamide increases twitch and tetanus tension in chicken fatigued slow muscle and during metabolic inhibition. These effects could be mediated by an increase in the calcium release from sarcoplasmic reticulum.

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Insulin Resistant Skeletal Muscle: Mitochondria Structure, Dynamics And Calcium Homeostasis

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Oxidative metabolism operated by mitochondria is altered in insulin resistant skeletal muscle, and these alterations are crucial for phenotype development. Mitochondria interconnected network is required for cell calcium homeostasis, whereas calcium modulates oxidative metabolism as well as mitochondria fission and fusion process. Skeletal muscle fibers, present two subpopulations of mitochondria: subsarcolemmal (SSM) and intermyofybrillar (IMF) mitochondria. SSM electron chain activity is decreased in insulin resistant and diabetic patients; moreover, mitochondria fusion protein Mfn2 is repressed in obese, hyperinsulinemic rats. We evaluated the structure-function relation between mitochondria dynamics and excitation-contraction coupling (ECC) associated calcium transients.

We developed an insulin resistance animal model based on high fat feeding of C57BL/6J mice and we studied mitochondria form skeletal muscle fibers. Proteins associated to mitochondria fission machinery, Drp-1 and Fis1 presented elevated levels in total muscle extracts from insulin resistance animals, as well as particular subsarcolemmal accumulation, evaluated using inmunohistochemistry. Furthermore, we developed an image processing method to quantify mitochondria network continuity in both SS and IMF mitochondria.

When we analyzed calcium transients associated to ECC in flexor digitorum brevis (FDB) fibers, we found a differential effect of insulin. Fibers from control animals, presented a decrease in calcium transients amplitude after cells were treated with insulin; however, fibers from insulin resistant animals, presented unaltered calcium transients.

Taken together, these evidences indicate that there is a differential distribution of mitochondria fission machinery proteins in control and insulin resistant skeletal muscle fibers, and that ECC associated calcium transients show differential susceptibility to insulin in control versus insulin resistant fibers that may be due to altered mitochondria structure adaptation.

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Sarcoplasmic Reticulum Calcium Content in Normal and Dystrophic Mammalian Skeletal Muscle Fibers

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The evaluation of the maximal amount of releasable Ca^{2+} (Ca^{2+}-content) that is stored in the sarcoplasmic reticulum (SR) of skeletal muscle fibers is a topic of great importance in muscle physiology. We have developed a method to quantitatively estimate the SR Ca^{2+} content in isolated mammalian muscle fibers based on Ca^{2+} dependent fluorescence measurements performed in the presence of high concentrations of Ca^{2+} buffers, and model calculations of Ca^{2+} flux underlying the fluorescence changes. Single fibers were enzymatically isolated from murine FDB muscles and impaled with two microelectrodes. One electrode, filled with 1M KCl, was used to record the membrane potential throughout